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# Association Mapping of Root Traits for Drought Tolerance in Bread Wheat

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Israr Ahmad, Niaz Ali, Habib Ahmad and Inamullah

Additional information is available at the end of the chapter

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## Abstract

Bread wheat (*Triticum aestivum* L.,  $2n = 6x = 42$ , AABBDD) is one of the most important crops, making staple food for more than 40 countries and over 35% of the global population. Drought stress is among the major constraints to wheat production as it affects plant growth, gene expression and yield potential of the crop. Development of elite wheat cultivars with the ability to grow and reproduce in water-limited soils seems to be the most enduring solution of addressing drought stress. A total of 100 lines including well-adapted wheat cultivars were evaluated for important root traits and complemented with 102 PCR-based markers aiming to understand their genetic structure and to identify molecular markers that are closely associated to quantitative trait loci (QTLs) of important root traits. Alleles per locus are counted and polymorphic information content (PIC) values are calculated. Population structure of these lines was analyzed with general linear model (GLM) and mixed linear model (MLM) approaches for identification of QTLs associated with important root traits. The results indicated the presence of two novel QTLs on the homoeologous group 2 and group 5 of wheat that may be related to drought stress resistance. Our results may facilitate the development of agronomically desirable drought stress-resistant wheat germplasm.

**Keywords:** bread wheat, genetic variation, drought tolerance, association mapping, QTL

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## 1. Introduction

Wheat (*Triticum* spp.) is one of the most important and widely cultivated crops with the annual yield of 694 million metric tons. More than 40 countries and over 35% of the world population use wheat as their staple food [1, 2]. Wheat is cultivated on larger area than other

cereals and modified to different climatic conditions [3, 4]. Bread wheat ( $2n = 6x = 42$ ) and durum wheat ( $4x = 28$ ) are the two common cultivated species around the world. Bread wheat supplies about 95% wheat globally, while durum and other wheats (emmer ( $4x = 28$ ), einkorn ( $2x = 14$ ) and spelt ( $6x = 42$ )) provide only 5% of the world wheat [2, 5]. Human population is increasing rapidly and is estimated to reach 9.4 billion by 2050. Therefore, food production will require a greater yield from the present cropland without horizontal expansion [6]. Population growth, environmental pollution and utilization of croplands for other purposes may reduce the croplands by 10–20% [7]. To meet the growing demand of global food shortage of 2050, total food production must increase by 50% at least to meet out demands of 2050. Among the crop plants, wheat is an economic and rich source of energy and proteins and supplies one fifth of all human calories for the world population [8]. Plant breeders are always trying to find wheat germplasm having desirable traits such as tolerance to diseases and other abiotic stresses [7, 9]. There is no doubt that cereals such as wheat, rice and maize are the world's leading food crops for all humans and are the principal resources that have led to the emergence of human civilization.

## 2. Global wheat production

Wheat is one of the most important cereal and staple food crop around the world. It ranks first due to its area and production and contributes more calories to the world's human diet than any other crop. On the other hand, wheat also maintains its first rank among major cereals due to its higher protein and gluten contents [8–10]. In 1986–1987, the wheat production across the world, which was 521 million metric tons, was increased to approximately 572 million metric tons in 2005–2006 from an area of 220 million hectares [11] and 694 million metric tons in 2011–2012. In 2011, the European Union (137 million tons) was top ranking in wheat production countries followed by China (118 million tons) and the United States of America (54 million tons). Further, Canada, Australia, India, Pakistan and Argentina contribute about 79% of the total wheat production. The world trade market was very feasible for wheat in 2011, and 129 million tons of wheat was traded in the world market [12].

## 3. Drought stress

Drought is defined as water deficiency in the root zone of crops that result to decrease in yield during the plant life cycle [13]. The capability of a plant to grow and reproduce in water-limited area is referred to as drought tolerance. Drought stress is changeable in its intensity, length and effectiveness, and crop plants are required not only be able to survive, but their ability to produce a harvestable yield under drought stress is of practical importance [14]. Drought tolerance is a quantitative trait, influenced by complex phenotype and genetic interactions. Understanding the genetic basis of drought tolerance in crop plants is a prerequisite for developing superior genotypes. High temperatures, radiation, water and nutrient deficiencies are commonly encountered under normal growing conditions also pose somewhat similar challenges. Further, certain soil properties such as composition and structure can also affect the balance of these different stresses; see, *for example*, [15, 16].

Drought is the main environmental problem that causes high negative effect on cereal crops particularly wheat. During drought conditions, plants show a wide range of behaviors varying from great sensitivity to high tolerance [17]. Seasonal cyclic drought has great involvement in reduction of wheat, barley and other cereal yields [18]. Drought stress greatly affects plant growth, gene expression, distribution, yield and quality of crop in arid and semiarid areas around the world [19]. About 60% of crop production around the world is from arid and semiarid regions. The rate of rainfall critically fluctuates in these areas. In developing countries 37% of wheat is commonly grown in drought susceptible areas [20]. The major constraint to wheat production around the world is inadequate supply of water. Within the United States of America alone, about 67% of crop losses over the last 50 years have been due to drought. The 2012 drought in the United States of America was the worst in the last 60 years, and more frequent occurrences of water shortages are expected due to climate projections and increasing competition for water among urban, industrial and agricultural demand.

The plants' reaction to drought stress depends on plant growth (development), stress period and plant genetics [21, 22]. Drought can also influence morphophysiological features of plant such as growth, anatomy, morphology, physiology (stomatal closure, low photosynthesis, transpiration rate), biochemistry and ultimately productivity [23, 24]. Yield is the basic criteria for cultivation of crop varieties under drought conditions. Therefore, it is a great challenge for crop breeders to produce cultivars having good potential of survival in stressed (drought, salinity, cool) environment [14, 15, 25]. Breeding for drought tolerance is further complicated by the fact that several types of abiotic stress can challenge crop plants simultaneously. Further, given the complexity of drought tolerance, marker-assisted selection has not contributed significantly to cultivar improvement, and breeding for dry environments has relied on direct phenotypic selection. However, recent technological advances and the great potential in wheat to ensure sustainable food production have driven research programmes to improve this crop genetically despite the size and complexity of the genome. Nonetheless, drought tolerance breeding may be effective if the marker-assisted selection-based molecular linkage maps for crop species are available [15, 26].

### 3.1. Drought stress in Pakistan

Diverse climatic and soil conditions are available for wheat growing in Pakistan. About one third of the total land area comes under rain-fed regions where rainfall is unusual [27]. Drought and salinity are very common around the world and are among the most serious problems to the agriculture in Pakistan [28]. Arid and semiarid regions of the world are badly affected by water stress, and as result crop production is reduced. Irrigated areas sometimes face drought conditions due to inadequate supply of water and canal closures [23, 29]. Drought-tolerant varieties are those, where grain yield is least affected by drought stress, or drought-tolerant crops are those that take up maximum amount of water and lose minimum of water during dry conditions [1–5, 30]. To ensure high crop production in rain-fed areas, different aspects of agriculture like holding precipitation, reducing evapotranspiration and sowing of drought tolerant varieties are important. Wheat varieties cultivated in rain-fed areas of Pakistan are usually low yielding as well as pests and diseases that are susceptible but are well adapted and flourish in dry conditions. Still, the need to increase yield to meet the demands of growing population to ensure food security requires well-integrated efforts. Although global water scarcity may be an abstract concept to many and a reality for others.

But with no confusion, it is the result of myriad environmental, political, economic and social issues. The current global climatic conditions are to hit Pakistan, and therefore, the search for diverse and drought-tolerant sources of crop plants is of paramount significance to feed its ever-growing population. Marker-assisted selection is a cry of the day for yield improvement in drought stress areas of Pakistan. Thus, the use of molecular markers for tagging of drought resistance genes is needed [14, 15, 31].

#### 4. Materials and methods

During the current study, 100 wheat lines (**Table 1**) including well-adapted wheat cultivars were evaluated for important root traits. A total of 102 PCR-based markers were applied aiming to understand their genetic structure and to identify molecular markers that are closely associated to quantitative trait loci (QTLs) of important root traits (**Table 2**). Plant germination, DNA extraction and PCR profiling followed previously published standard procedures [32]. Further, population structure of these lines was analyzed with general linear model (GLM) and mixed linear model (MLM) approaches using TASSEL software with their default setting for identification of QTLs associated with important root traits.

S. no	Genotypes	S. no	Genotypes
1	Sonalika	2	Shalimar 88
3	Merco 2007	4	Khyber 83
5	Manther	6	Chenab 70
7	Lr-230	8	Soghat 90
9	Ksk	10	Pari -73
11	Maxi pak	12	Chakwal 86
13	Indus 79	14	Wadanak 98
15	Bakhtawar 94	16	Nori -70
17	Wadanak 85	18	ZA-77
19	Abdaghar 97	20	Kaghan 93
21	Margalla 99	22	Dawar 96
23	Uqab 2000	24	Suliman 96
25	Raskoh	26	AS-2002
27	Haider 2002	28	LYP-73
29	Local white	30	Noshera 96
31	MH-97	32	Sindh 81
33	Zarlashta 90	34	Fakhri sarhad
35	Punjab-76	36	10737

S. no	Genotypes	S. no	Genotypes
37	Faisalabad 85	38	10776
39	Barani 70	40	10748
41	Rawal 87	42	10724
43	NIAB 83	44	10792
45	GA 2002	46	Pirsabak 2008
47	Chenab 79	48	Punjab-96
49	Saleem 2000	50	Mumal-2002
51	Zamindar-80	52	SA-42
53	Iqbal-2000	54	Marwat-01
55	SH-2003	56	Barani-83
57	Anmol-91	58	Potohar-93
59	LU-26	60	Kohinoor-83
61	Chenab-96	62	Potohar-70
63	Faisalabad-83	64	Pak-81
65	Zarghoon-79	66	Pirsabak-85
67	C-228	68	C-273
69	Shahkar-95	70	Tandojam-83
71	Punjab-88	72	Dirk
73	10793	74	Bahalwapur-79
75	Punjab-81	76	Lasani-08
77	C-591	78	Sussi
79	Sutlag-86	80	Khyber-79
81	C-250	82	FPD-08
83	Blue silver	84	Sandal
85	RWP-94	86	Kiran
87	Sariab-92	88	Wardak-85
89	Wafaq-2008	90	Meraj-08
91	10742	92	C-518
93	010724-YR	94	potohar-90
95	AUP 5000	96	Mehran-89
97	WL-711	98	Janbaz
99	SA-75	100	AUP-4008

**Table 1.** List of wheat lines and cultivars used in the current study.

Marker	Marker	Marker	Marker	Marker	Marker
<i>Cfd</i> 15	<i>Xbarc</i> 154	<i>Xwmc</i> 232	<i>Xgwm</i> 372	<i>Xwmc</i> 177	<i>Xgwm</i> 293
<i>Cfd</i> 18	<i>Xbarc</i> 158	<i>Xwmc</i> 233	<i>Xgwm</i> 389	<i>Xwmc</i> 181	<i>Xgwm</i> 299
<i>Xwmc</i> 24	<i>Xbarc</i> 159	<i>Xwmc</i> 235	<i>Xgwm</i> 443	<i>Xwmc</i> 182	<i>Xgwm</i> 302
<i>Xwmc</i> 25	<i>Xbarc</i> 163	<i>Xwmc</i> 398	<i>Xgwm</i> 471	<i>Xwmc</i> 216	<i>Xgwm</i> 325
<i>Xwmc</i> 27	<i>Xbarc</i> 164	<i>Xwmc</i> 420	<i>Xgwm</i> 469	<i>Xwmc</i> 219	<i>Xgwm</i> 359
<i>Xwmc</i> 43	<i>Xbarc</i> 165	<i>Xwmc</i> 606	<i>Xgwm</i> 484		
<i>Xwmc</i> 51	<i>Xbarc</i> 167	<i>Xwmc</i> 718	<i>Xgwm</i> 544		
<i>Xwmc</i> 52	<i>Xbarc</i> 172	<i>Xwmc</i> 749	<i>Xgwm</i> 608		
<i>Xwmc</i> 94	<i>Xbarc</i> 173	<i>Xwmc</i> 798	<i>Xgwm</i> 609		
<i>Xwmc</i> 97	<i>Xbarc</i> 175	<i>Xbarc</i> 42	<i>Xgwm</i> 642		
<i>Xwmc</i> 104	<i>Xbarc</i> 264	<i>Xbarc</i> 45	<i>xgwm</i> 908		
<i>Xwmc</i> 147	<i>Xgwm</i> 4	<i>Xbarc</i> 76	<i>Xgdm</i> 3		
<i>Xwmc</i> 149	<i>Xgwm</i> 10	<i>Xbarc</i> 101	<i>Xgdm</i> 5		
<i>Xwmc</i> 153	<i>Xgwm</i> 33	<i>Xbarc</i> 127	<i>Xgdm</i> 6		
<i>Xwmc</i> 154	<i>Xgwm</i> 37	<i>Xbarc</i> 128	<i>Xgdm</i> 19		
<i>Xwmc</i> 157	<i>Xgwm</i> 55	<i>Xbarc</i> 134	<i>Xgdm</i> 28		
<i>Xwmc</i> 161	<i>Xgwm</i> 60	<i>Xbarc</i> 137	<i>Xgdm</i> 33		
<i>Xwmc</i> 163	<i>Xgwm</i> 71	<i>Xbarc</i> 140	<i>Xgdm</i> 46		
<i>Xwmc</i> 166	<i>Xgwm</i> 99	<i>Xbarc</i> 141	<i>Xgdm</i> 114		
<i>Xwmc</i> 167	<i>Xgwm</i> 111	<i>Xbarc</i> 144	<i>VRN</i> AF		
<i>Xwmc</i> 168	<i>Xgwm</i> 136	<i>Xbarc</i> 147	<i>VRN</i> B1 R3		
<i>Xwmc</i> 169	<i>Xgwm</i> 194	<i>Xbarc</i> 148	<i>PpD1</i> R1		
<i>Xwmc</i> 175	<i>Xgwm</i> 261	<i>Xbarc</i> 149	<i>PpD</i> 1 R2		

**Table 2.** List of PCR primers/molecular markers used in the current study.

## 5. Root trait analysis and its significance to drought

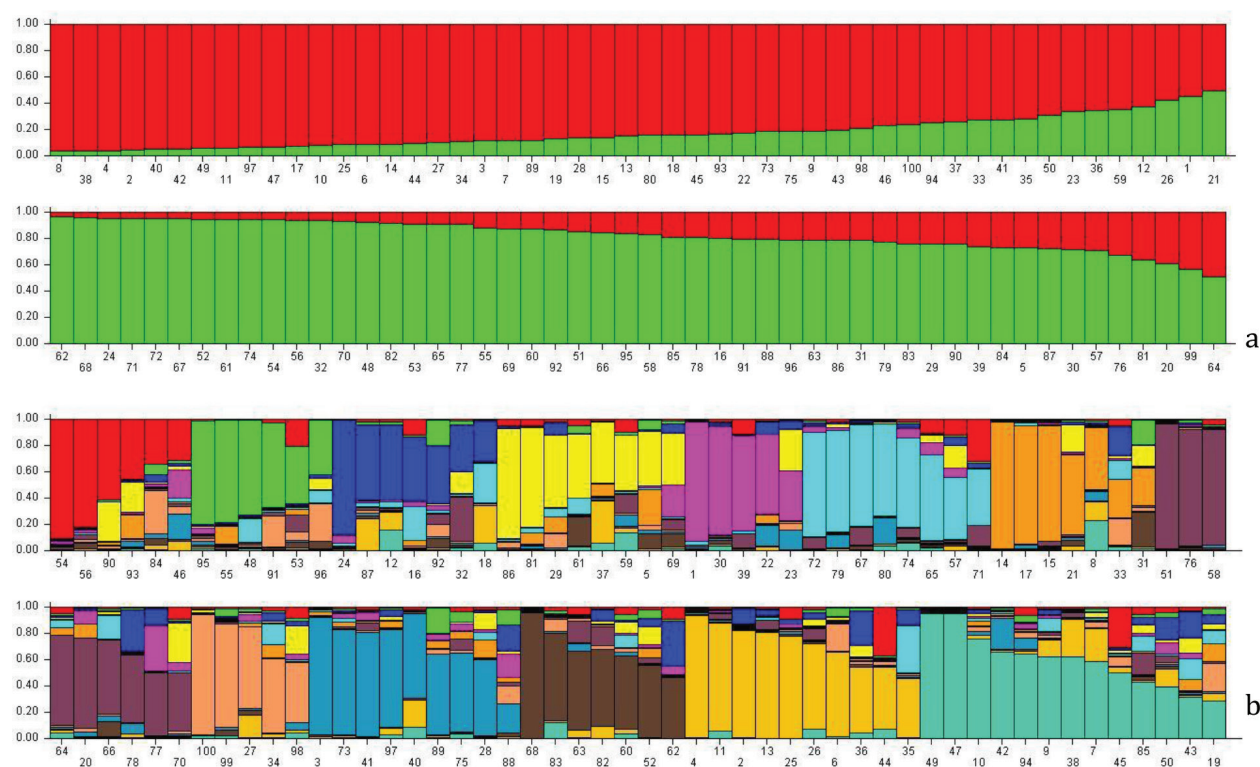
To understand the performance of wheat crop under drought conditions, it is necessary to have a sound knowledge about root traits. Root traits vary from species to species on the base of water availability, growth, physiology and architecture [33]. Root surface area and root length in wheat crop play an important role in water uptake. A well-organized root system is necessary for efficient water uptake. In crops, fibrous root system consists of two types as seminal and nodal roots [34]. Well-developed root system could play positive role in water deficit (drought) areas. Root morphological traits greatly affect water and nutrient uptake. Herbaceous plants with fine roots, smaller diameter and greater root length are better adapted to dry conditions [35]. Root traits greatly influence the resource uptake and sustaining crop yield under drought stress conditions. For maximum grain yield in wheat, active and well-developed root system is necessary [36, 37].



## 6. Association mapping between root traits and SSR markers

In the present study, association mapping was applied for identification of association between root traits and SSR markers. Marker-trait association (MTA) based on polymorphism found in SSR markers applied on diverse wheat genotypes. Two different models were used for identification of QTLs associated with root traits as GLM (general linear model) and MLM (mixed linear model). GLM requires no kinship, and only Q matrix was used to determine association between markers and mean of phenotypic traits. The level of significance of P value was measured at  $p \leq 0.01$  in both GLM and MLM models. The QTLs having LOD values above 2.5 were considered for both GLM and MLM.

A sum of 102 molecular markers were used in the present study. Most of the markers showed high level of polymorphism. A total of 271 polymorphic alleles were generated. The alleles per locus ranged from 1 to 3 and an average of 2.63 per locus. Polymorphic information content (PIC) values of the markers were also calculated in the range of 0.03–0.59. Initially, in order to investigate the genetic diversity of the material, 100 wheat genotypes were grouped into different cluster populations (**Figure 1**). Population structure may lead to spurious association between marker and traits [38]. Therefore, a model-based approach was used for association mapping. Both the general linear model (GLM) and mixed linear model (MLM) were applied. The association analysis also concluded that hundreds of genotypes having different genetic backgrounds were classified into 13 distinct groups, viz., G1, G2, G3, G4, G5, G6, G7, G8, G9, G10, G11, G12 and G13.



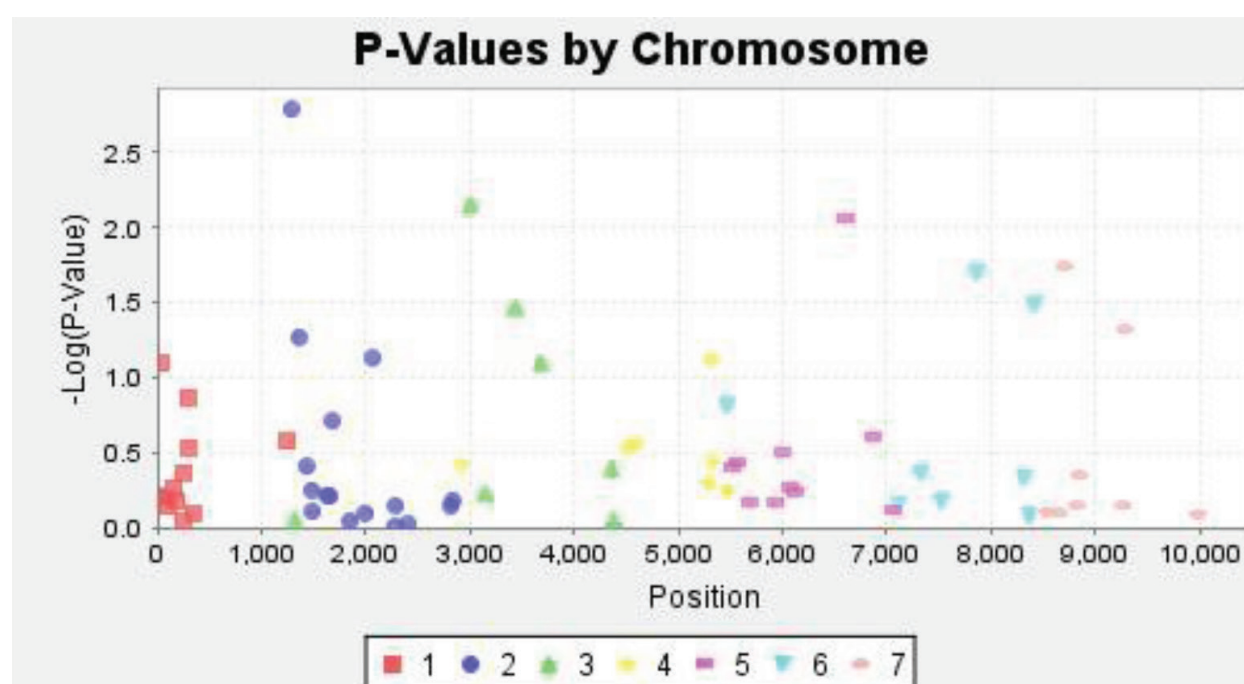
**Figure 1.** Population structure analysis of wheat genotypes based on SSR markers. (a) Graphical bar plot at  $k = 2$  presenting two subgroups (G1 and G2). (b) Graphical bar plot at  $k = 13$  presenting 13 subgroups (G1–G13). The X-axis shows accession numbers, and Y-axis shows subgroup membership.



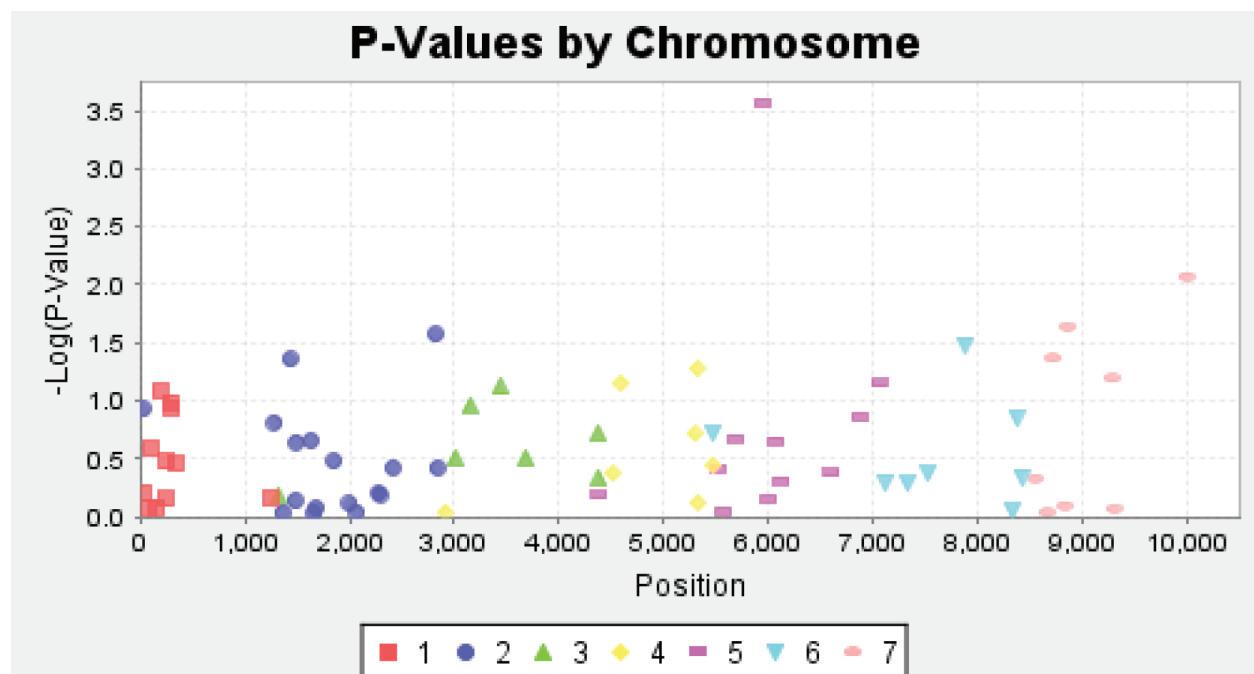
### 6.1. Total root length

Total root length per unit ground area ( $L_a$ ) is often considered to be directly related to the amount and rate of water uptake. Total root length (TRL) is associated with drought tolerance in wheat because it marks the spreading of roots in the soil and affects the resource uptake [39]. The genotype Pirsabak-85 ranked high on the base of TRL and R:S and considered to be the best for drought tolerance by extracting water stored in the deep soil layers. Further, in GLM model the SSR marker *Xgdm 5* on chromosome 2 was significantly associated with total root length, but no association of marker with TRL was found in MLM. The phenotypic variance ( $r^2$ ) was 0.10. The p value was recorded as 0.0016, and LOD is 2.78 as shown in **Figure 2**. The present research revealed that GLM model confirmed MTA for TRL was found to be located on chromosome 2D and the results are in accordance with previous results where MTA for TRL was reported on chromosome 2 at 3.4 cM [40].

For root fresh weight, the GLM model identified MTA associated with RFW, located on chromosome 5B. The marker *Xwmc 235* attributed to trace the QTL on specific chromosome for RFW. The phenotypic variance ( $r^2$ ) was found as 0.10, and LOD was 3.56 as shown in **Figure 3**. The previous report of Ayman, A.D., M. A.M. Atia, H.A. H. Ebtissam, A.H. Hashem and S.A. Sami. 2013. A multidisciplinary approach for dissecting QTL controlling high yield and drought tolerance-related traits in durum wheat. *Int. J. Agri. Sci. Res.* 3: 99-116 confirmed that four QTLs are associated with RFW located on 2B, 5B, 6A and 6B chromosomes. Our results which did not localize other QTLs due to lesser number of markers have been used. Similarly, for root dry weight, the *PpD1* marker revealed marker trait association (MTA) for RDW in GLM model only. The MTA was found to be located on chromosome 2A having  $r^2$  0.41 and LOD of 2.7 as in **Figure 4**. These results were partly in agreement with results of [41] where the authors found that 5 QTLs for RDW were grouped in chromosomes 2A and 7A.



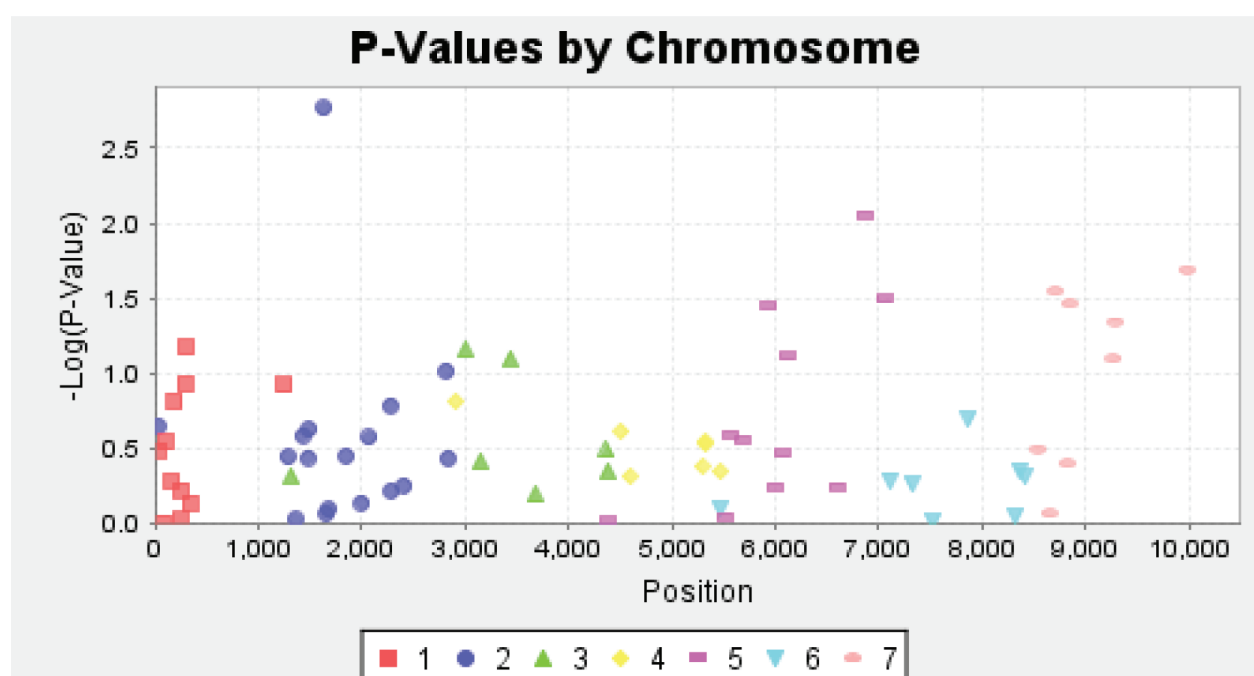
**Figure 2.** QTL identified for TRL on the basis of LOD in GLM.



**Figure 3.** QTL identified for RFW on the basis of LOD in GLM.

## 6.2. Maximum root length

The maximum root length (MRL) evolved to capture deeper water from the soil under drought stress [42]. The Abdaghar-97 genotype recorded the maximum root length (MRL) to capture deep soil moisture in dry areas. Two MTAs were identified for MRL located on chromosomes 2A and 5B. MTA of chromosome 2A was marked by *Xgwm 10* having LOD (2.68) and that of 5B was



**Figure 4.** QTL identified for RDW on the basis of LOD in GLM.

attributed by *Xwmc 149* having LOD of 2.86 as in **Figure 5a** and **b**. So far only one QTL for maximum root length located on chromosome 4B has been reported [14]. Similarly, QTL was identified for MRL located on chromosome 5 at 158.5 cM [43]. Therefore, the MTA identified on 2A chromosome in the present study was not reported before and considered to be novel QTL for MRL.

6.3. Number of nodal roots

The bulk of roots would increase with the increase in number of tillers. Nitrogen uptake is affected by length and number of nodal roots [44]. The uptake of nutrients is 2–6 times

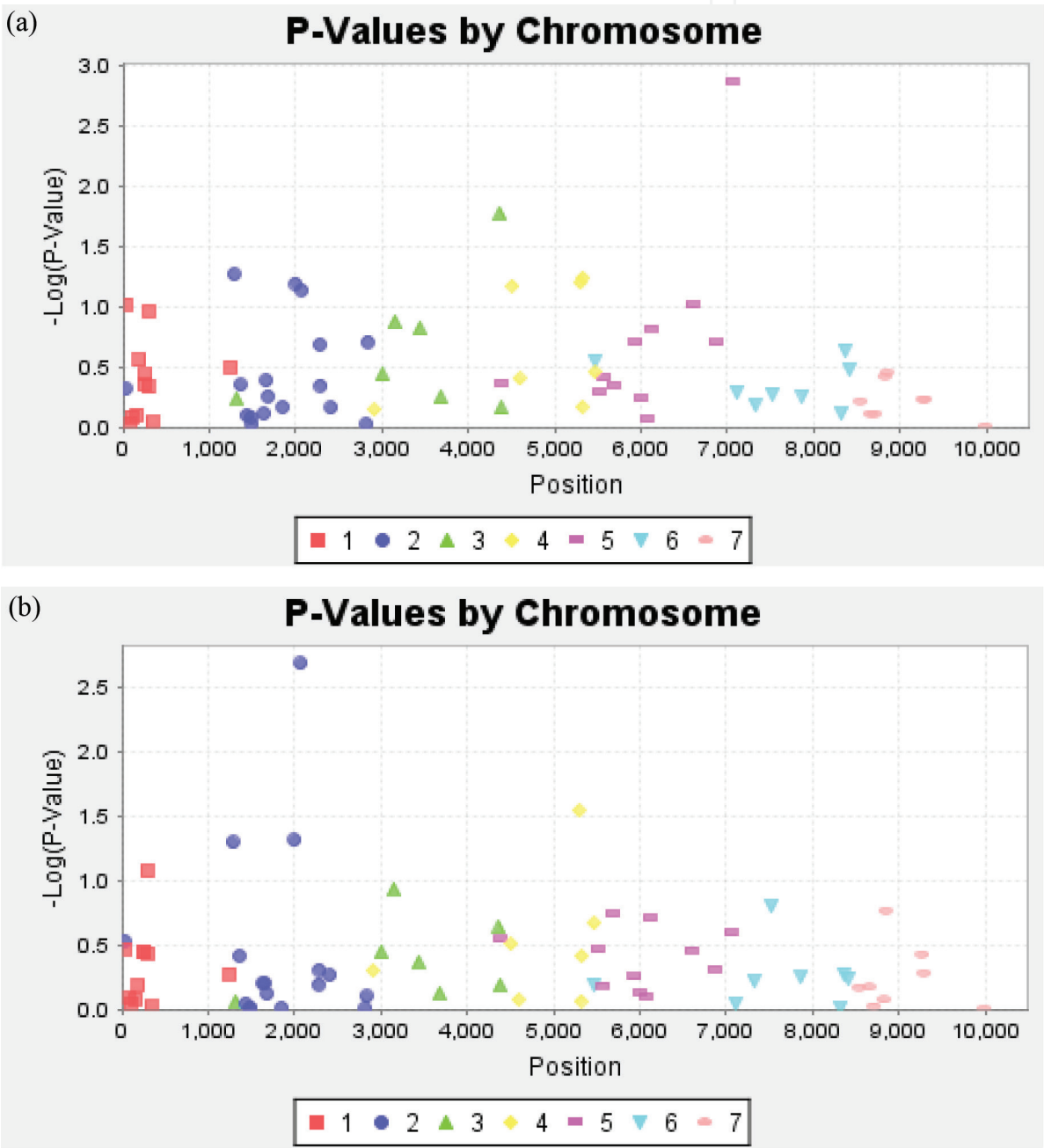
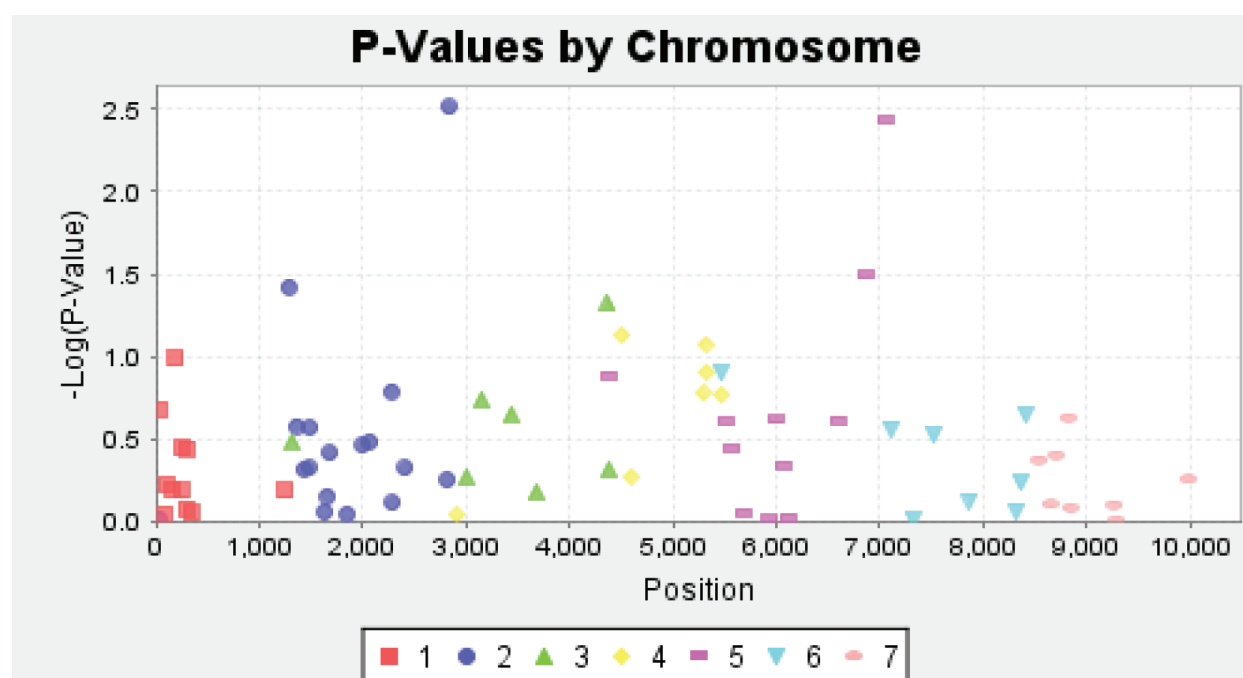


Figure 5. QTL identified for MRL on the basis of (a) GLM; (b) LOD in MLM.

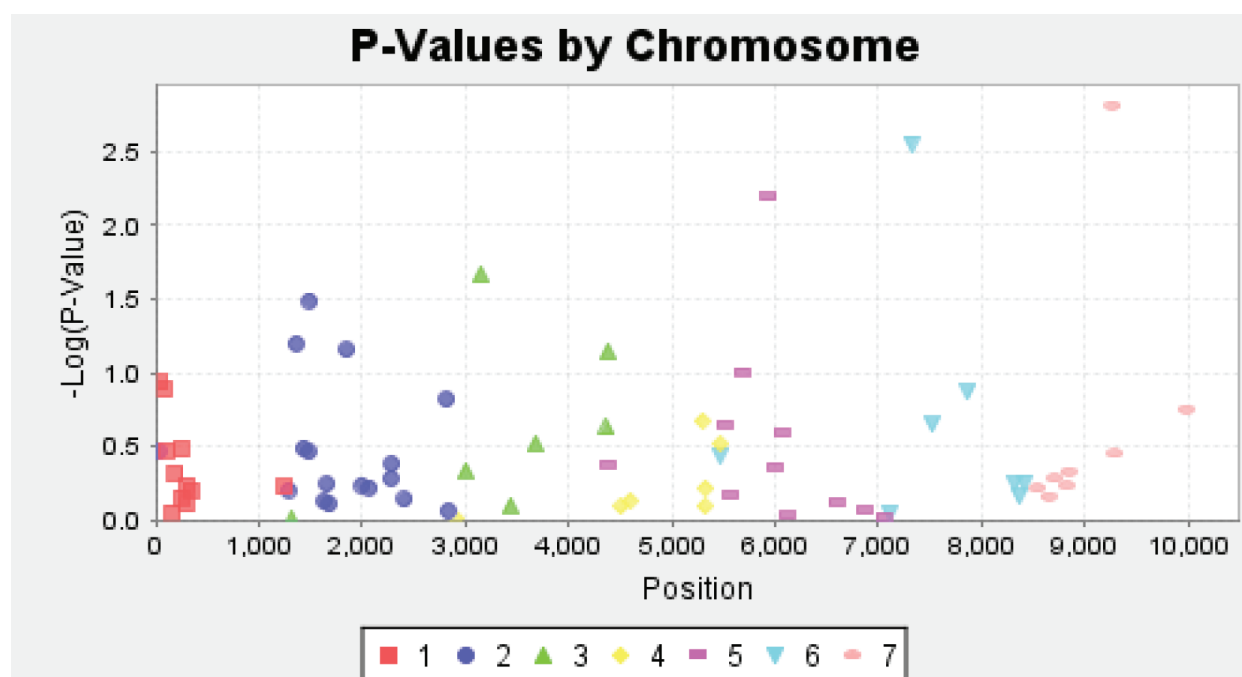
more for nodal roots than seminal roots, and thus growing such genotypes in rain-fed areas would be desirable [45]. The results of the present study found Meraj-08 with high number of nodal roots and would be better for nitrogen and water uptake in rain-fed areas. As for as the number of nodal roots MTAs was concerned, the MTA for number of nodal roots located on chromosome 2B. SSR marker *Xwmc 175* recognized the MTA for NNR on chromosome 2B as shown in **Figure 6**. MTA for NNR was found at LOD 2.5,  $p$  value 0.00306, while the ( $r^2$ ) 0.17. Our results were accordance with result of [46] where the same QTL is reported on chromosome 2B. Two MTAs (QTLs) were found associated with root angle in GLM model. The MTAs were found to be located on chromosomes 7B and 6D. The MTA located on chromosome 7B recognized by *Xgwm 302* and that of 6D was identified by *Xwmc 749* as in **Figure 7**. The results are consistent with previous results where QTL for RA was located on chromosome 7B at 86cM, and reported four QTLs for RA was located on chromosome 2A, 3D, 6A and 6D [47, 48].

#### 6.4. Root density

Root density (RDT) increases the efficiency of the root system and is considered to be the most important trait for uptake of phosphorus in wheat [42]. The genotype Soghat-90 ranked first on the base of RDT and is considered to be good for phosphorus uptake. Further, root density has been reported to be positively correlated with total root length, root diameter and water use efficiency [49]. Two MTAs were identified for root density (RDT) in both GLM and MLM models located on chromosomes 2B and 5B. The MTA for chromosome 2B was attributed by *Xwmc 175* and 5B by *Xwmc 235* having LOD of 3.28 and 2.5 as in



**Figure 6.** QTL identified for NNR on the basis of LOD in GLM.

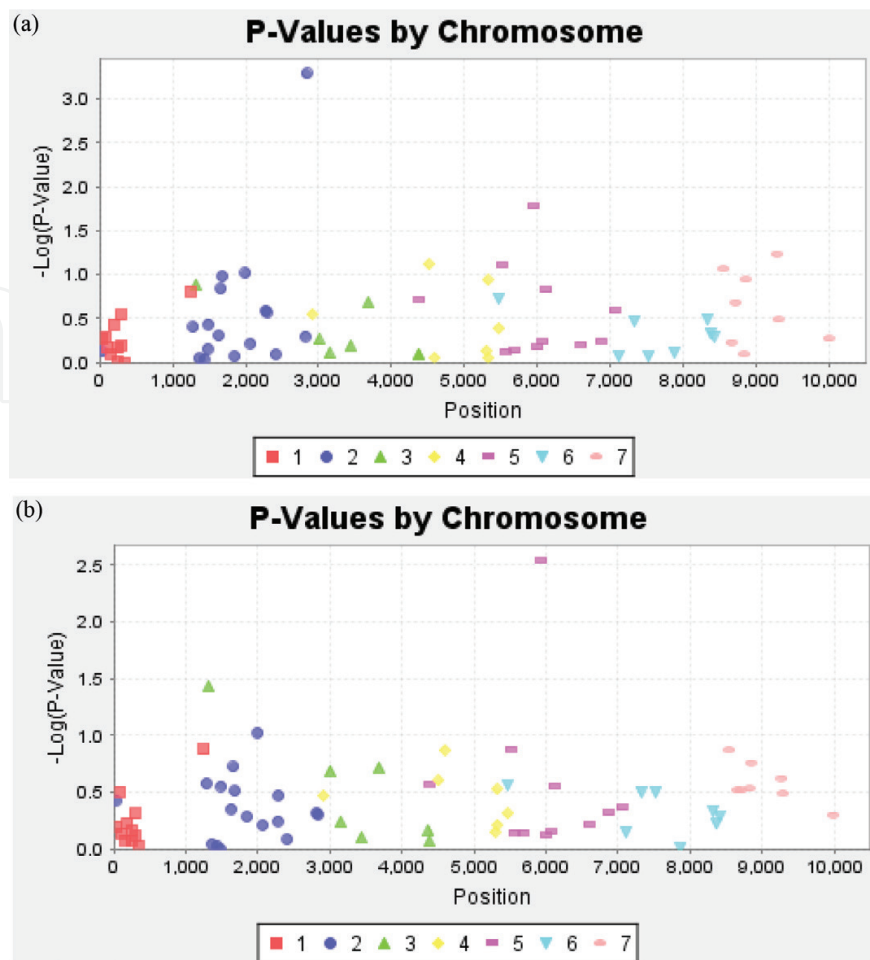


**Figure 7.** QTL identified for RA on the basis of LOD in GLM.

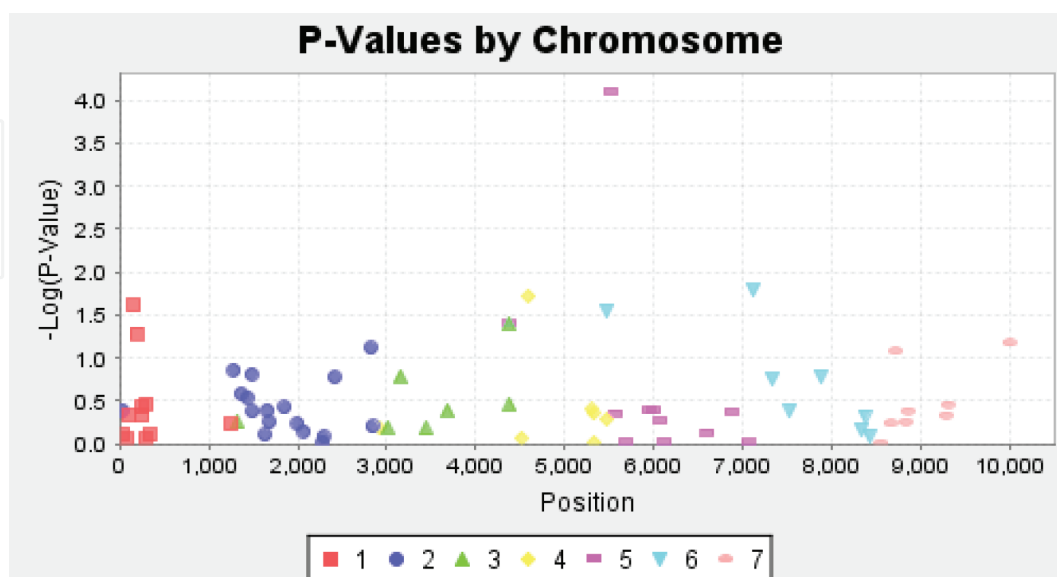
**Figure 8a and b.** The results of the present study are in accordance with the earlier reports. Previously QTL for RDT has been reported on chromosome 2B at 158.5 cM and 5B at 47 cM [46, 50]. The number of seminal roots may result in better adaptation to drought conditions in wheat. Further, the number of seminal roots was negatively correlated with water use efficiency [51, 52]. The strong root system will reduce the WUE and hence will reduce biomass production. Therefore, it is needed to improve the root system function rather than a strong root growth for wheat survival in drought conditions. In the present study, the genotype Marwat-01 is recorded the highest NSR and is suggested to be good in more water uptake in rain-fed areas.

### 6.5. Root diameter

The high root diameter (RD) is associated with drought tolerance in wheat. The genotypes showing the highest RD are supported for drought stress tolerance due to large xylem vessels with increased resource uptake and are well organized in searching deep soil layers to extract water [53]. Further, total root length, maximum root length and root density increase or decrease extremely with a small change in root diameter and decrease in root diameter would increase crop yield under drought. Significant reduction in root diameter, total root length and root density under drought conditions has been previously documented [37, 54]. Two MTAs were identified for RD, each in GLM and MLM. Both MTAs were located in chromosome 5B, attributed by *Xwmc 233* having LOD 3.1 and 3.3 as in **Figure 9**. Our results were consistent with earlier reports, where QTLs for RD on chromosome 5B at 4.5 cM have been mentioned [55].



**Figure 8.** QTL identified for RDT on the basis of LOD in (a) GLM; (b) MLM.



**Figure 9.** QTL identified for RD on the basis of LOD in GLM.



## 7. Conclusion

Among the abiotic stresses that limit wheat crop productivity, drought stress alone is by all means one of the most devastating factors. In the past, breeding efforts to improve drought tolerance response have been hindered primarily by its quantitative nature as well as our poor understanding of the physiological basis of yield in water-deficient conditions [16]. So far, most QTLs for drought tolerance in wheat have been identified through yield and yield component measurements under water-limited conditions. No doubt, yield is the most desirable trait to breeders; still, it is very difficult to relate water use efficiency and identify potential target regions for positional cloning [15]. Only few studies have associated QTLs with specific components of drought response. Although the development of gene-based molecular markers and genome sequencing should accelerate positional cloning, the genomic regions associated with individual QTL are still very large and are usually unsuitable for breeding programme [51–55]. From an application point of view, it is imperative to select genotypes that are able to optimize water use efficiency while maximizing yield in response to drought. Improving the competence of root systems to extract water from the soil is highly desirable, and any extra water extracted during grain filling definitely remarkably increases the yield in wheat. Thus, identification of markers or genes associated with root growth and architecture would be particularly useful for breeding programmes to improve root traits by molecular marker-assisted selection.

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## Author details

Israr Ahmad<sup>1\*</sup>, Niaz Ali<sup>2</sup>, Habib Ahmad<sup>3</sup> and Inamullah<sup>3</sup>

\*Address all correspondence to: iabotany32@gmail.com

1 Department of Botany, Women University Azad Jammu & Kashmir Bagh, Pakistan

2 Department of Botany, Hazara University Mansehra, Pakistan

3 Department of Genetics, Hazara University Mansehra, Pakistan

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